

eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of the vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer $10^{0.7}$ times greater than that used in the immunogenicity test, but not less than $10^{2.0}$ titration units (PFU or ID₅₀) per dose.

(4) *Identity.* Bulk or final container samples of completed product from each serial shall be tested for identity as prescribed in paragraph (b)(3) of this section and shall meet the criteria stated therein.

[50 FR 438, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 64 FR 43045, Aug. 9, 1999]

DIAGNOSTICS AND REAGENTS

§§ 113.400–113.405 [Reserved]

§ 113.406 Tuberculin, Intradermic.

Tuberculin, Intradermic, is a filtrate produced from cultures of Pn, C, and Dt strains of *Mycobacterium tuberculosis* (supplied by Animal and Plant Health Inspection Service) which has been inactivated and is non-toxic. Each serial shall be tested for purity, safety, potency, and special chemical tests in accordance with the conditions prescribed for each test. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Each serial shall be tested for purity as provided in this paragraph.

(1) Final container samples of completed product shall be tested for viable bacteria and fungi as prescribed in § 113.26.

(2) A 20 ml sample shall be centrifuged and the sediment examined microscopically for the presence of acidfast (Ziehl-Nielsen stain) or other microorganisms (Gram stain). A serial which contains microorganisms is unsatisfactory for release.

(b) *Safety test.* Final container samples of completed product from each serial shall be tested for safety. Two mature guinea pigs shall be injected

subcutaneously with 1 ml and observed for 10 days. If unfavorable reactions attributable to the product occur during the observation period, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and repeated: *Provided*, That if the test is not repeated, the serial shall be declared unsatisfactory.

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be subjected to a comparison test using a Reference Tuberculin supplied by Animal and Plant Health Inspection Service. Test animals shall be 10 sensitized white female guinea pigs from one source which weigh 500–700 grams at the beginning of the test and which have not been used in a previous test. The comparison test shall be conducted in accordance with the procedures prescribed in paragraphs (c)(1), (2), (3), (4), (5), (6), (7), and (8) of this section.

(1) The guinea pigs shall be sensitized with a sterile heat-killed suspension of equal amounts of strains Pn, C, and Dt of *Mycobacterium tuberculosis*. The heat-killed sensitizing agent shall be injected in a volume of 0.5 ml per guinea pig. The guinea pigs shall be considered sensitized for testing not less than 30 days nor more than 120 days post-injection.

(2) The guinea pigs shall be prepared for sensitivity testing at least 4 hours prior to the injection of tuberculin. The entire abdominal and flank areas shall be clipped, a depilatory agent applied for 5–10 minutes, the area rinsed with warm water, and dried.

(3) Dilutions of 1:100, 1:200, and 1:400 shall be prepared with the Reference Tuberculin and the unknown tuberculin. Three test sites on each side of and equidistant from the abdominal midline shall be chosen on each guinea pig. Using a tuberculin syringe and needle, 0.05 ml of each dilution shall be injected intradermally at one of the test sites which has been randomly selected for the dilution.

(4) The sensitivity of the tuberculin shall be determined 24 hours after injected by measuring the area of erythema. Measurements in millimeters shall be made anterior of the greatest diameter and perpendicular to the first

measurement. The square millimeter shall be calculated by multiplying the two measurements.

(5) The total area of response for each tuberculin tested shall be determined by adding the areas of erythema for each dilution of each of the test animals in a group. The sums of the areas of erythema for all three dilutions of each tuberculin shall be added to give the total area of tuberculin response.

(6) The total tuberculin response area of the serial being tested shall be expressed as a percentage of the total tuberculin response area of the Reference Tuberculin. (The total response area of the serial divided by the total response area of the Reference Tuberculin times 100.)

(7) If the total tuberculin response area of the serial being tested does not fall between 75 percent and 125 percent of the total tuberculin response area of the Reference Tuberculin, the serial is unsatisfactory.

(8) Two unsensitized guinea pigs are given 0.05 ml intradermal injections of 1:4 and 1:10 dilutions of both the serial being tested and the Reference Tuberculin as a control for nonspecific positive reactions. If positive reactions are observed with the Reference Tuberculin, the test is considered a "No Test" and repeated. If positive reactions are observed with the serial being tested only, the serial is unsatisfactory.

(d) *Special chemical tests and requirements.* Final container samples of completed product from each serial shall be tested as follows:

(1) *Hydrogen ion concentration.* The hydrogen ion concentration shall be determined with a pH meter which has been standardized with a pH 7.0 buffer just prior to use. The pH of the product shall be 7.0 ± 0.3 .

(2) *Total nitrogen determination.* The nitrogen content shall be determined by the Kjeldahl method on duplicate 15 ml samples consisting of 5 ml from each of three vials. The total nitrogen content of the product shall be 0.18 percent ± 0.06 percent.

(3) *Trichloroacetic acid precipitable nitrogen.* The determination of precipitable nitrogen by a final concentration of 4 percent trichloroacetic acid shall be made by the Kjeldahl method on du-

plicate 15 ml samples, consisting of 5 ml from each of three vials. The trichloroacetic acid precipitable nitrogen content shall be 0.047 percent ± 0.01 percent.

(4) *Phenol determination.* The phenol content shall be determined by direct titration with a standardized bromide-bromate solution. (A correction factor of 0.04 should be subtracted from the final value in the determination of phenol in tuberculin.) The phenol content shall be 0.54 percent ± 0.04 percent.

(5) *Clarity.* The product shall be optically clear and free from any extraneous particles.

[39 FR 16857, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974. Redesignated at 55 FR 35561, Aug. 31, 1990, as amended at 56 FR 66784, Dec. 26, 1991]

§ 113.407 Pullorum antigen.

Pullorum Antigen shall be produced from a culture of representative strains of *Salmonella pullorum* which are of known antigenic composition, high agglutinability, but are not sensitive to negative and nonspecific serum. Each serial shall be tested for purity, density, preservative content, sensitivity, homogeneity, and hydrogen ion concentration. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product shall be tested for viable bacteria and fungi as prescribed in § 113.26. In addition, each serial shall be free from extraneous organisms as determined by Gram staining and microscopic examination.

(b) *Nephelometric determination of bacterial density.* The bacterial density shall be 80 ± 15 times McFarland No. 1 standard for stained antigen K's and 50 ± 10 times McFarland No. 1 standard for tube antigen.

(c) *Preservative requirements.* (1) The formalin content of Pullorum Stained Antigen K shall be 1.0 ± 0.2 percent as determined by a colorimetric method.

(2) The phenol content for Pullorum Tube Antigen shall be 0.55 ± 0.05 percent as determined by direct titration with a standardized bromide-bromate solution.

(d) *Sensitivity requirements.* (1) Each serial of antigen shall be compared